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Effect of *Hugonia mystax* Leaves on Histopathological Studies, Tissue Glutathione and Lipid Peroxide Levels of Rat Liver

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Abstract:- The present investigation was aimed to determine hepatoprotective potential of ethanol leaf extract of *Hugonia mystax* (HMEE). 100 mg/kg silymarin and 200 and 400 mg/kg p.o HMEE were administered to the Group III to Group V respectively. Antioxidant parameters tissue glutathione (GSH) and lipid peroxidation (LPO) levels and histopathological observations are assessed. *in vivo* antioxidants studies and Histopathological findings established liver protection effects of HMEE.

Keywords:- *Hugonia mystax, Ethanol, Hepatoprotective, Antioxidant.*

I. INTRODUCTION

Liver contribute in most of the biochemical pathways which involve in fight against disease and energy provision etc.(Ward and Daly, 1999).

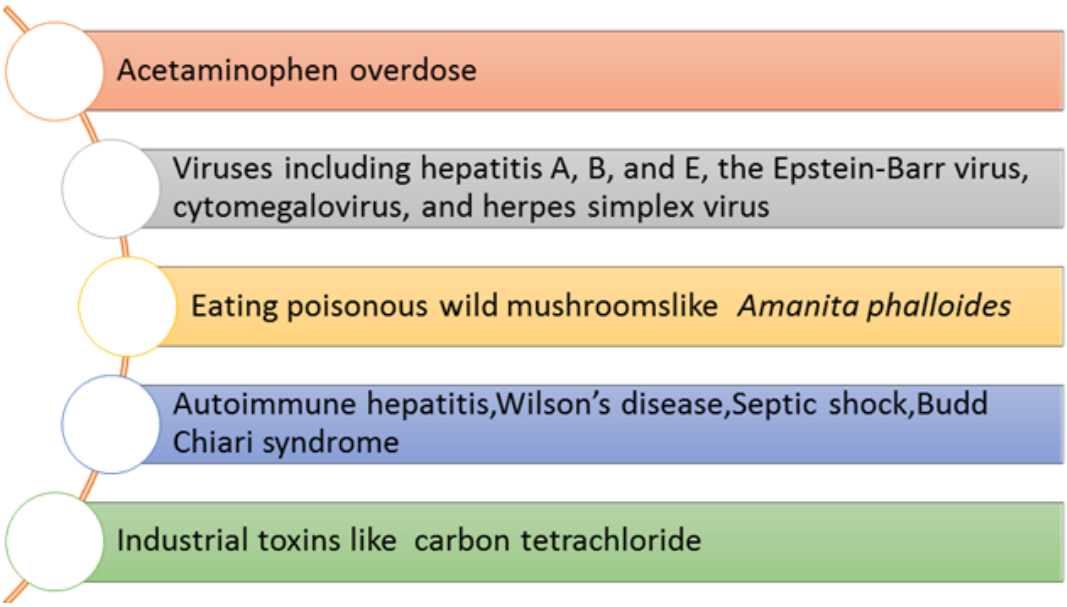

Fig 1:- Causes of Acute Liver Failure or Liver Toxicity

Repetitive use of numerous therapeutic agents like paracetamol, anti-tubercular drugs, agrochemicals and various food preservatives are hostile for liver toxicity as shown in above Figure 1.

Further addiction of alcohol and other drugs aggravated the problem and malnutrition which is major cause of liver damage. Modern medications along with plant based formulations and various nutraceutical have little to intention for improvement in hepatic or liver diseases (Karan *et al.*, 1999; Chaterrjee, 2000).

Hugonia mystax is a rambling scandent scrub belongs to family Linaceae. Leaves are simple, penninerved and alternate (Kirtikar & Basu, 1999). Literature survey reveals that roots are anthelmintic, astringent, antidote and anti-inflammatory (Vaidyaratnum, 1995, Nadkarni, 2002, Mohankumar *et al.*, 2015). The current literature reveals that plant possess antimicrobial activity (Vimalavady *et al.*, 2012), anti-inflammatory activity (Rajeswari *et al.*, 2013) and cytotoxic effect (Anandakumar *et al.*, 2011).

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Preliminary phytochemicals analysis of *Hugonia mystax* ethanol extract (HMEE) revealed the presence of polyphenolic compounds. There are documentary proof of numerous polyphenolic components are showing potential hepato-protective action (Tiwari, 2001). Hence, the aim of the study was to determine hepato-protective potential of HMEE against hepatotoxicity in rats.

II. MATERIALS AND METHODOLOGY

A. Plant Material & Preparation of HMEE

Plant taxonomist Dr. Chetty provided authentication data of *Hugonia mystax plant*. The ethanol (70% ethanol) extract was prepared using de-fatting process. Preliminary phytochemical tests indicated the existence of saponins, flavonoid and tannin in 70% ethanol extract of *Hugonia mystax* leaves (HMEE). So, HMEE was employed to evaluate of hepatoprotective activity.

Fig 2:- *Hugonia mystax* Leaves

B. Experimental Animals

Wistar albino mice weighing between 18-25 g and rats weighing between 150-220g were used. The experimental design was approved from the institutional committee (1554/PO/a/11/CPCSEA) as per the Committee for the Purpose of Control And Supervision of Experiments on Animals (CPCSEA) guidelines.

C. Acute Toxicity Studies

Guideline for Testing Of Chemicals i.e OECD Guide line no 420 which used for Acute Oral Toxicity the acute toxicity,which was utilized for determination on albino mice (Veeraraghavan, 2000). For this study 200 mg/kg ,400 mg/kg doses were used on the basis no mortality at dose 2000 mg/kg.

D. Hepatoprotective study [Kapoor et al., 1994; Gulati et al., 1995.]

Healthy wistar albino rats were alienated into 05 groups each consist of 06 animals.

Group	Name of the Group	Administration for 21 days
Group I	Normal control group	Distilled water (5 ml/kg body weight, p.o) as vehicle
Group II	Intoxicated group/ ethanol treated group	40 % ethanol (2 ml/100g body weight, p.o.)
Group III	standard group/ silymarin treated group	Silymarin (100 mg/kg body weight, p.o.) and 40 % ethanol (2 ml/100 g p.o.).
Group IV	HMEE treated group	HMEE (200 mg/kg body weight, p.o.) and 40 % ethanol (2 ml/100 g p.o.)
Group V	HMEE treated group	(400 mg/kg body weight, p.o.) and 40 % ethanol (2 ml/100g p.o.)

Table 1

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E. Histopathology: -

The liver tissue was isolated from the Wistar albino rats and normal saline used for washing. The 10% buffered neutral formalin solution was used for storage of liver which further used for histopathological study.

F. Determination of Invivo Tissue Glutathione

Ellamn method (Aykae *et al.*, 1985) was used to perform Tissue Glutathione measurements. Liver tissue sample (1 gm) was added in ice cold trichloroacetic acid (10 ml) to homogenize liver tissue in tissue homogenizer. The prepared mixture was centrifuged and collected 0.5 ml supernatant. Disodium hydrogen phosphate solution [2ml, 0.3 M] and dithiobisnitro benzoate solution [0.2 ml (0.4mg/ml, 1% sodium acetate)] was added in 0.5 ml supernatant. The absorbance of prepared sample was noted at 412 nm.

G. Determination of Invivo Lipid Peroxidation (Buege and Steven, 1978)

The biological sample (1 ml) was mixed to TBA-HCL-TCA solution (2.0 ml). The prepared mixture was heated for one hour. After cooled the mixture solution, precipitate was collected by centrifugation process. The absorbance of prepared sample was noted at 535 nm.

H. Statistical Analysis

The one-way ANOVA followed by Turkey-Kramer multiple comparisons test was employed for Statistical analysis for Results. The results of GSH and LPO levels were stated as mean ± SEM (n=6).

III. RESULTS AND DISCUSSION

There were a significant (P<0.05) rise in LPO and diminution of GSH level in ethanol intoxicated group. Treatment with standard Silymarin at dose100 mg/kg augmented tissue GSH level by 31.55 % and decreased tissue LPO level by 53.35 % inhibition. Treatment with HMEE restored GSH levels (figure 3). The HMEE treatment expressively (P<0.05) reduced the lipid peroxidation (figure 4).

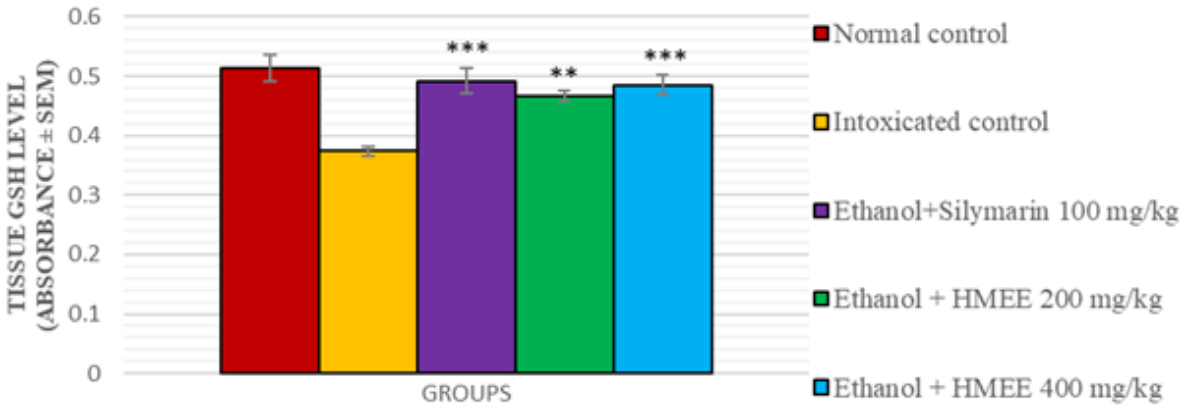

Fig 3:- Effects of Treatment on Tissue GHS Levels in Ethanol Induced Liver Damage in Rats

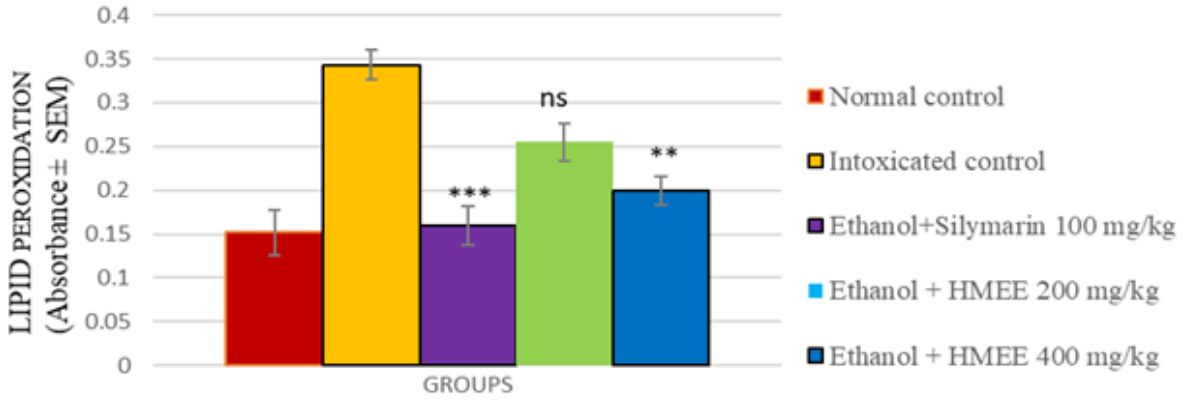

Fig 4:- Effects of Treatment on Lipid Peroxide Level in Ethanol Induced Liver Damage in Rats

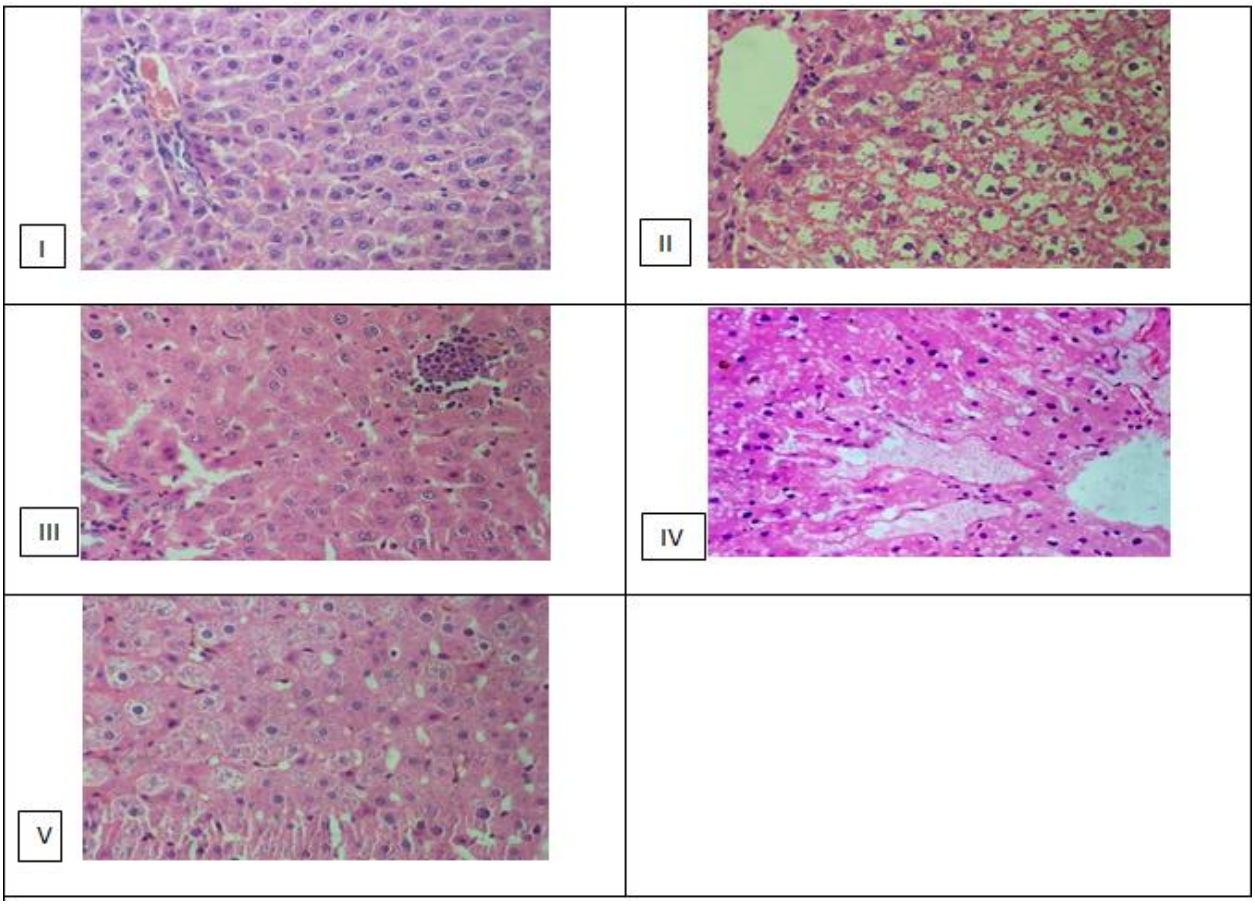

Fig 5:- Histopathological studies. Group I. Normal control group presentating normal liver structural composition include normal liver lobules, kupffer cells and central vein. Group II Intoxicated group showing disturbed liver architecture includes severe degeneration of hepatocytes. Group III. Standard drug group showing maintained liver architecture, normal Hepatocytes and minimal inflammatory cells. Group IV. HMEE treated group (Ethanol + HMEE 200 mg/kg) showing slight enlargement of sinusoids and vacuoles and liver architecture is partially disturbed. Group V. HMEE group (Ethanol + HMEE 400 mg/kg) showing normal sinusoids and slight enlargement of hepatocytes (H & E 40X)

IV. DISCUSSION

The numerous mechanisms/paths are responsible to cause liver dysfunctions in rats. The first mechanism is chronic alcoholism which increase the release of endotoxin that activates Kupffer cells. This leads hypermetabolic state and hypoxia in Liver organ. This phenomenon causes formation of toxic free radicals/components upon reestablishment of oxygen and triggering cell death (Adachi *et al* 1994; 1995).

Second mechanism; ethanol is converted in acetyl aldehyde. Liver tissue contains XDH protein i.e xanthine dehydrogenase cytosolic enzyme converted into xanthine oxidase [Brass *et al.*, 1991]. Acetyl aldehyde is catalyzed by Xanthine dehydrogenase/xanthine oxidase into acetate which generates reactive oxygen species.

Overall, both mechanisms are responsible for hepatotoxicity (Kennedy and Tipton, 1990, Albano *et al.*, 1988). Hepatic hypoxia which is commonly cause by Ethanol further portrayed as a hepatotoxicity (Gulati *et al.*, 1995).

V. CONCLUSION

HMEE possess significant *in vivo* antioxidant and hepato-protective activities. It may be due to presence of saponins and flavonoids. Future detailed molecular level study is desirable for isolation and characterization for hepato-protective activity.

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